A BACTERIAL ROSETTE DISEASE OF LETTUCE

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[1]In cooperation with the College of Agriculture, Ohio State University, Columbus.
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BULLETIN
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A BACTERIAL ROSETTE DISEASE OF LETTUCE

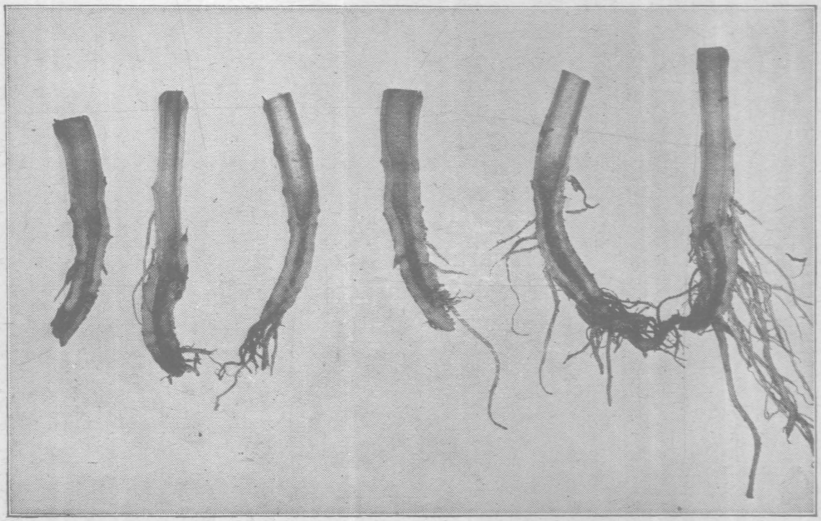
ROY C. THOMAS

A preliminary report of a lettuce disease new to the State was given in the monthly bulletin of this Station issued January, 1920. It was thought at that time that the organism causing the disease was a bacterium probably identical with one previously found in South Carolina, studied and reported by Miss Nellie Brown[1] of the United States Department of Agriculture. Further investigations, however, aided by a comparative study with a type culture of *Bacterium vitians* have revealed characteristics both of similarity and difference between the organism found in South Carolina and that found in Ohio. This report comprises a more detailed study of the cultural characteristics of the Ohio organism.

In November, 1919, we were asked to investigate a lettuce disease which was causing concern in a greenhouse in central Ohio. The plants in the beds were 7 weeks old, developing very unevenly, with a tendency to rosette. This condition of retarded growth was accompanied generally by a yellowing or flaccid condition of the outer leaves. During days of bright sunshine when the temperature of the house became higher, the slight wilting or somewhat flaccid appearance of affected plants was more noticeable, followed by a recovery of turgidity during the night. The soil throughout the beds was uniformly watered and the moisture content was adequate at all times. The sub-soil, composed of sand and gravel, provided ample drainage for any excess accumulation of water. Cultural conditions maintained in the house were excellent. It seemed apparent that some parasite must be the primary cause of the irregularity of development and pathogenic condition. This

[1]Brown, Nellie A., Some Bacterial Diseases of Lettuce. Jour. Agl. Research, Vol. XIII, No. 7, pp 367-388, pl 29-41, 1918.

was confirmed later by inoculation work. This form of retarded development or rosette may well be mistaken for infections caused by the fungus Rhizoctonia, the general appearance of the diseased plants being very much the same in both cases. Since later investigation has revealed the presence of the bacterial rosette in all districts of Ohio where lettuce is grown under glass, it is thought that the disease has been present for some time in the State and has escaped detection due to the fact that it was assumed to be identical with the older recognized type of rosette. This likewise was the assumption under which the present investigation was begun. However, when detailed examination failed to reveal the

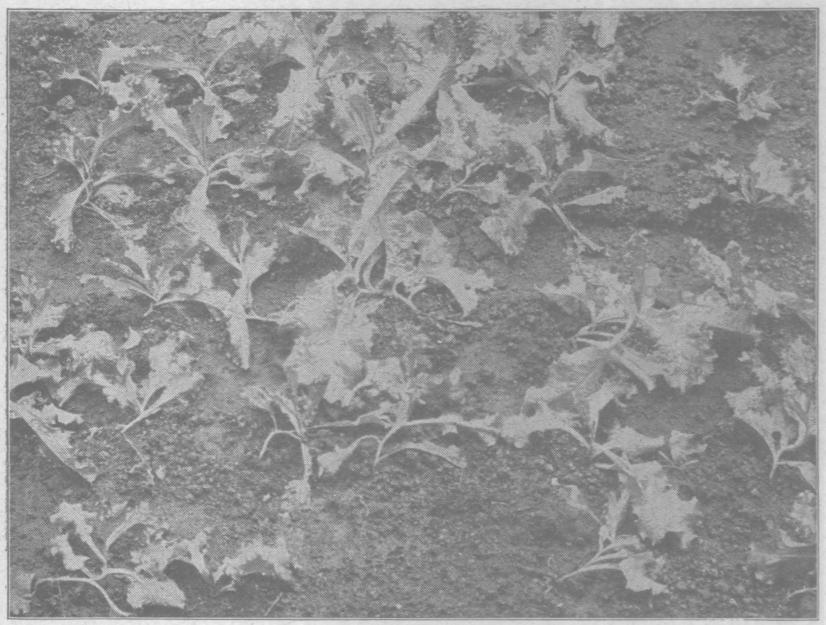


Stems of lettuce cut longitudinally showing the type of infection produced in the bacterial rosette disease

presence of hyphae characteristic of Rhizoctonia, and when in our poured-plate nutrient, agar-agar cultures made from macerated fragments of diseased plant tissue taken under aseptic conditions from the interior of the roots and stems of diseased plants, there was always consistently found a number of colonies apparently of a single species of bacterium, it was thought probable that the disease might be of bacterial origin. Inoculation work later demonstrated the pathogenicity of the organism isolated.

For convenience this disease of lettuce will be designated as a bacterial rosette to differenciate it from the type of rosette caused by the fungus rhizoctonia.

Losses caused by the bacterial rosette disease have never been observed to be as severe as those frequently encountered in cases of Rhizoctonia infections, yet they have been found to vary from a trace to 60 percent. Losses must be calculated in terms of reduced poundage, of arrested development, and delayed maturity. Plants infected in the seedling stage never mature sufficiently for marketing. On the other hand, if infection does not occur until after plants have been reset into the larger beds, a fairly satisfactory crop may be obtained, yet the period of maturity may be extended from 1 to 3 weeks. Although no difficulty has been experienced in



Bed of lettuce 10 weeks old grown in infected soil

detecting by cultural methods the presence of the bacteria in the diseased tissues of affected plants, they are not readily demonstrated in cross sections of living material. Bacteria could not be demonstrated with certainty in stained cross-sections, yet cultural examinations never failed to reveal their presence. A microscopic examination of longitudinal and cross-sections of the stems and roots reveals a yellowish or brownish substance in portions of the vascular system, particularly the xylem. This substance was noted to be soluble in alcohol and acetic acid. The color in 10 to 12-week old plants which have become badly rosetted, is dark

brown and may extend throughout the entire stem; yet usually is not found to reach as far as the petioles of the lower leaves in living plants, and then only when accompanied by heavy secondary infections in plants which have succumbed. In plants 6 to 8 weeks old the invasion of the bacteria upward in the stem appears to be from one-half of an inch to an inch above the surface of the ground. This may be roughly discerned by the yellow to brownish color of the vascular system. It is thought that a yellow coloration only is developed by the reaction of the bacteria upon the plant tissues, and that secondary saprophytic organisms are likely responsible for other color gradations of varying shades of brown. It has been noted in inoculation work under definite control that only the yellow color of the vascular system was produced.

Two lettuce plants, 10 weeks old, grown in infected soil, showing development, which is ordinarily attained by healthy plants within 4 or 5 weeks

The root system is most seriously affected. The small fibrous roots are apparently first attacked at their tips and very soon cease to function. The infection slowly progresses upward until the larger roots are invaded. The larger roots continue to function partially and endeavor to restore the impoverished system. These very readily become detached when the plant is pulled from the soil. In this way infectious material accumulates very rapidly. No difficulty has ever been experienced in isolating the pathogene from dead rootlets or from the soil clinging to diseased plants. If the soil is carefully removed from diseased plants the dead rootlets, very much browned, will often be found still intact.

The bacterium has never been observed to cause a rot or spots upon the leaves or stems of lettuce plants, and has never been found to be a rot-producing organism. In all instances when a suspension of the bacteria was sprayed upon leaves of healthy lettuce plants, results were negative. The chief role of the organism seems to be to attack the root hairs and small fibrous roots of the lettuce plant, gain entrance into the vascular system and interfere with the free passage of food material.

The bacterial rosette disease has been found only associated with the Grand Rapids variety. Since this is the only variety grown in commercial houses in Ohio no attention has been given to the heading types, in the inoculation work which follows.

INOCULATION WORK TO DETERMINE PATHOGENICITY OF ORGANISM

All inoculation work was conducted with sub-cultures of a single colony.

The first series of experiments in this connection was made during the winter of 1919 in the departmental hothouse or pathologium. The soil in the beds was not sterilized. Two crops of lettuce of the Grand Rapids variety had been previously grown and in each case careful examination had been made for the presence of symptoms of the disease under investigation. Since no lettuce disease could be detected further preparation of the soil was not considered necessary.

The lettuce seedlings were started in flats and as soon as old enough to be handled easily were transplanted once before setting into the larger beds. When four to five weeks old certain of the flats were inoculated from 48-hour bouillon cultures of the organism by pouring the liquid on the surface of the soil around the seedlings, which were allowed to remain undisturbed for about a week longer. Other flats were left uninoculated as checks. Later the seedlings from both inoculated and uninoculated flats were transplanted, respectively, to larger beds. Care was taken in the case of the inoculated seedlings to remove a goodly portion of soil with each plant and to disturb the roots as little as possible.

For the first two weeks after setting no difference could be noted in the development of check and inoculated plants. At the close of the third week, however, it was evident that the plants in the check beds were developing more rapidly than the others. This difference became steadily more marked from week to week and was very striking at the close of the ninth week, when the crop in

the check beds was sufficiently developed for marketing. At this time the inoculated plants were developed unevenly, with 60 percent showing varying degrees of rosette, 20 percent with spindling type of growth, and the remainder at a stage of development ordinarily attained by healthy plants after a growing period of six weeks. An examination of all of the inoculated plants revealed that the root systems were badly attacked and that the organism had gained entrance into the stem in nearly all cases, manifested by a yellowing and browning of the tissues, especially of the vascular portion. There was no decay except where secondary organisms were associated with the pathogene.

Lettuce plants 7 weeks old. Left and right diseased plants; middle, disease free plant. The deficient root system is characteristic of diseased plants

Cultures were made from the stems of 10 plants and also from the soil adhering to the roots. The organisms isolated proved to be identical in cultural characteristics and reaction toward stains, with a sub-culture from the original colony used for inoculation.

The same beds were twice reset with lettuce plants of the same age as those used in the first experiment. One test was conducted in the spring of 1920 and the other in the autumn of the same year.

No further inoculation of the soil was made, and in each case disease-free plants were used. In both instances the disease of lettuce was reproduced in the inoculated soil, while the check beds remained disease free. The flaccid condition and wilting of the leaves of rosetted plants during the day was more marked, due to the difficulty in keeping the temperature of the house down during the spring and fall months.

In the mid-winter season of 1920 another experiment was made to determine the effect of the inoculum in the soil upon the older lettuce seedlings. The plants used were eight weeks old and had been grown at a low temperature. They had a well developed root system. They rooted after transplanting and continued to grow quite uniformly with little evidence of disease, and developed a very satisfactory crop. The check bed, however, matured and was harvested the ninth week after setting, whereas the diseased beds did not show the same stage of maturity until the twelfth week. Examination of the roots showed a much smaller degree of infection than in former cases, and only a slight, yet distinct, yellowing of the interior of the stem. The organism, nevertheless, was recovered from diseased rootlets and from the lower portions of the stems in 7 out of 10 trials.

A series of 10 lettuce plants six weeks old was used to determine the ability of the bacterial rosette organism to cause spots upon leaves or to attack any portion of the lettuce plants above ground. Each plant was placed in a 6-inch pot and covered with a bell jar. Seven of the plants were sprayed with a well-clouded suspension of bacteria in physiological normal salt solution. In addition the leaf petioles of two plants were injured by pricking with a sterile needle. Two of the remaining plants were sprayed with the sterile salt solution, while the other was left unsprayed. The test was continued for two weeks. No spots developed on any portion of the leaves. The needle pricks became somewhat enlarged, slightly brown at the borders, yet there was no evidence of bacterial infection.

DESCRIPTION OF THE ORGANISM

The organism which causes this disease of lettuce is a bacterium, a short rod, rounded at the ends. No motility has been observed and all attempts to demonstrate the presence of flagellae have been negative. It occurs singly, in short chains of three or four, or longer chains of seven to ten, depending upon the medium on which it is grown. On potato starch agar, potato broth, potato

plugs, or nutrient broth made alkalin to 20 percent Fuller's scale it seems to develop most luxuriently, with the long chain formation very noticeable. After incubation from 24 to 48 hours, the length of individual organisms has been noted to vary from 1.4 to 1.9 u and the width .5 to .85 u in preparations stained with saffron, by Gram's method or in darkfield India ink preparations. When grown upon other media such as nutrient broth, plain nutrient agar, peptone broth, etc., the tendency to chain formation is less noticeable and the individual organisms are smaller, varying from .9 to 1.5 u in length and .4 to .8 u in width after an incubation period of 24 to 48 hours. In seven-day and older cultures the rods are very short, showing a near approach to the coccus type. No other variation in form has been found.

The bacterium is strictly aerobic. No growth occurs in cultures incubated under anaerobic conditions. The medium remains clear in the closed arm of a fermentation tube of the U-shaped type, or in Durham's modified form. In all liquid cultures heavy clouding is first manifest at the top, and if left undisturbed develops downward very slowly, scarcely extending more than 1 to 1½ centimeters from the top after three weeks incubation. Later, clumps or masses of bacteria form which sink to the bottom of the culture tube. In agar stab cultures slight development occurs beyond one-half a centimeter from the surface.

No endospores are produced. Nutrient broth cultures, 1 month, 6 months, and 10 months old were examined. In each case the viability of the cultures was first established. One cubic centimeter from each culture, well-shaken, was heated in a water bath at 60 degrees C for 10 minutes. No growth developed from transfers made after heating, to broth or to agar slants. Spore stains employed on preparations from the same cultures were uniformly negative.

Small clumps or masses composed of chains varying in length fall to the bottom of the tube in liquid cultures after four weeks or longer incubation. The pseudozoogloeae eventually settle to the bottom of the tube, more rapidly, however, if the culture is occasionally agitated. The sediment disintegrates very readily and quickly enters into suspension when the culture is shaken. This was noted to be characteristic of the sediment in all liquid cultures.

SPECIFIC CULTURAL CHARACTERS

The organism grows well upon all ordinary media employed in cultural work. Certain characteristic reactions are included with this report which will aid in identification of the species.

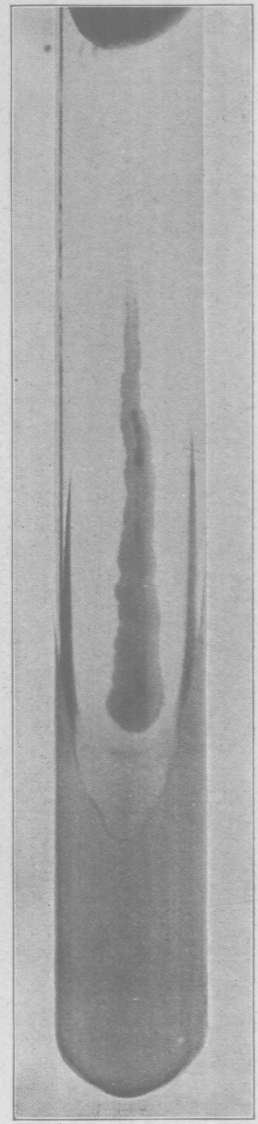
In peptonized beef bouillon with the reaction adjusted to +15 Fuller's scale, marked clouding occurs after 18 to 30 hours at the temperature 25 to 30 degrees C. Growth is heaviest at the top of the liquid where a delicate pellicle forms after 4 to 6 days, which very readily breaks into small particles when disturbed and settles to the bottom. The sediment en masse is yellowish in color. If allowed to remain undisturbed for two or three weeks, a rather heavy pellicle, pyrite yellow[2] in color, will develop, forming a ring upon the tube which adheres quite firmly.

Nutrient agar plates.—Colonies are visible within 24 hours when poured from a young bouillon or or potato plug culture, and incubated at a temperature 25 to 30 degrees. Surface colonies are round, entire, opaque, greenish yellow in color when examined against a dark background with a hand lens magnifying 14 to 20 diameters. The colony appears to be finely reticulate, somewhat raised, and glistening. After 36 to 48 hours the color is olive buff, and even for an indefinite period this color continues with slight change if the culture is kept in the dark, whereas if incubated in ordinary or subdued light the characteristic pyrite yellow color is developed. After three days the reticulate effect is scarcely discernible.

Nutrient agar slant.—Growth along streak is good, spreading or fan-shaped at the bottom. The entire surface of freshly prepared slants with adequate moisture present will be covered with the bacterial growth within 6 to 9 days at room temperature. Color reactions are constant as previously described. The color of the medium remains unchanged.

Nutrient agar stab.—Growth is abundant at the surface, yet extending along the line of the stab only 5 to 6 millimeters. No liquefaction of the medium occurs.

Bouillon over chloroform and formaldehyde.—The bacterium was tested to determine its ability to grow over chloroform and 40 percent formaldehyde. The test was terminated at the close of seven days with the result that no growth had occurred over chloroform on nutrient agar slants, nutrient bouillon or potato plugs, while fair growth had developed over formaldehyde but was evidently considerably retarded. The same cultures were allowed to stand under room conditions for four days after the test was discontinued. None of those



Steak culture 36 hours old on nutrient agar showing type of growth of organism

[2]The color determinations are according to Ridgway. Ridgway, Robt., Color Standards and Color Nomenclature. 43 p., col., pl., 1912.

which had been placed over chloroform made any growth, while those over formaldehyde made slight growth with slight yellow pigment developing.

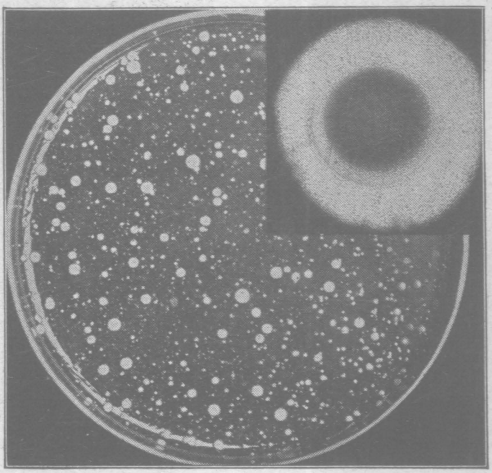
Nutrient gelatin stab.—In nutrient gelatin cultures +10 Fuller's scale incubated at 10 to 15 degrees, growth is good at the surface yet is feeble along the stab. Liquefaction is first crateriform beginning within 2 to 3 days and progressing slowly. After three weeks the medium is liquefied with the line of division curved slightly downward to a depth of 1 centimeter, and after six weeks to a depth of 1½ to 2 centimeters. When held for longer periods liquefaction made very slight advance and the loss of moisture due to evaporation inhibited further growth. The color is characteristic as on other media.

Sterilized potato plugs.—On freshly sterilized potato plugs growth after 3 to 4 days is abundant, smooth, shining, slightly viscid. The color reaction is constant, at first olive buff and continues so if incubated in the dark, changing when allowed to stand in the light to pyrite yellow.

Bouillon with sodium chlorid added.—Good growth was noted after 14 days in all flasks containing graduated percentages of sodium chlorid up to and including 7 percent. Only slight clouding occurred at 8 percent. No growth was apparent in medium to which 9 percent of sodium chlorid had been added.

Cohn's solution.—No growth.

Fermi's solution.—Good growth developed after 20 days, liquid straw yellow, rather heavily clouded with yellowish sediment in the bottom of the flasks, readily going into suspension. No pellicle formed, yet a delicate ring was evident upon the glass at the surface of the liquid. This soon disintegrated with agitation.



Dilution plate 48 hours old showing type of growth of surface and submerged colonies. Single colony x 500 times, 36 hours old. The opaque center and reticulate peripheral portion are quite characteristic

Uschinsky's solution.—The rate of growth after 20 days appeared to be the same as in Fermi's solution. The liquid was found to be pinkish buff to vinaceous buff in color. Considerable white sediment collected in the bottom of the flask, readily going into suspension. Pellicle was absent. An evanescent film was noted on side of flask.

Sterile cream-free milk.—Growth proceeded slowly at room temperature from the surface of the liquid downward. The curd separated after 7 to 10 days and later was slowly digested. The liquid gradually cleared and a series of color gradations occurred. The characteristic yellow pigment appeared in a ring which formed at the surface of the liquid upon the glass, when the tubes were allowed to remain undisturbed. After 11 days the color of the liquid became olive buff, after 28 days coral pink, after 37 days rufous. The last color-

change, to coral red, was noted after 58 days. This remained constant until the cultures became dried. As the cultures advanced in age a flaky sediment, readily disintegrating and entering into suspension when agitated, settled to the bottom. After 60 days the reaction of the cultures was found to be strongly alkaline. This was determined to be due to the formation of ammonia.

Sterile litmus milk.—Color reactions were masked by the litmus. The development of an alkaline condition of the media deepened the blue color of the litmus from the surface of the liquid downward indicating by color gradations the zones of greater and lesser growth of the organism.

OTHER CULTURAL CHARACTERISTICS

Ammonia.—The production of ammonia is quite strongly marked in milk and bouillon cultures and without special test may easily be detected in plate cultures two weeks old.

Nitrates.—Nitrate bouillon and nitrate peptone cultures varying in age from 24 hours to 3 weeks were tested. In all cases there was a strong reduction of nitrates.

Indol.—When the bacterium was grown in 1 percent peptone water cultures for 10 days there was a slight production of indol. Older cultures showed an appreciable increase.

Hydrogen sulphid.—All tests made for the production of hydrogen sulphid were negative.

DEGREES OF ACIDITY TOLERATED

The ability of the organism to grow in liquid media varying in degrees of acidity was determined for malic, tartaric, lactic, citric, and acetic acids. In media acidified with malic acid to +14 degrees Fuller's scale, good growth occurred, yet there was no clouding at +16 degrees. With tartaric acid growth developed at +20 degrees Fuller's scale, but none at +22 degrees. Media acidified with lactic acid titrating +12 degrees showed moderate clouding. None, however, occurred at +13 degrees. With citric acid at +13 degrees Fuller's scale there was good growth, but none at +15 degrees. When acetic acid was used good growth developed in media titrating +17 degrees Fuller's scale, but none at +20 degrees. The organism is apparently most sensitive to lactic acid.

DEGREES OF ALKALINITY TOLERATED

Sodium hydroxide was the only alkali used in this test. In bouillon cultures rendered alkaline to —20 degrees Fuller's scale with sodium hydroxide, dense clouding occurred after 10 days. There was none perceptible at —26 degrees. It is thought that

most luxuriant growth develops in nutrient broth made alkalin to points between —1.0 and —2.0 degrees, in which the organism attained the largest size noted and the long chain formation was especially marked.

LONGEVITY ON CULTURE MEDIA

Nutrient agar slant cultures made December 8, 1919, were allowed to remain under laboratory conditions without further addition of moisture or food material until November 17, 1920, when portions of the dried residue were transferred to fresh agar slants, and to tubes of broth. In all cases growth characteristic of the

Microphotograph of cross-section of diseased lettuce stem showing the invasion of the vascular bundles

organism developed. In another examination, the viability of a broth culture made April 12, 1920, was tested after one year. This likewise was found to be alive. It is evident that the organism will remain viable in both solid and liquid culture media for at least a year under ordinary laboratory conditions.

Potato starch.—In potato starch growth was good, but diastatic action feeble. In 1 percent peptone broth to which was added 1

percent, respectively, of each of the following sugars,—dextrose, saccharose, lactose, mannit, maltose, also glycerine, growth was good without the formation of acid or gas.

Sterile uncooked potato.—The object of this experiment was to determine whether the bacterial rosette organism was capable of growing upon, or causing a rot of, uncooked potato tubers. Five potatoes, disease free so far as could be determined by gross inspection were used. These were cleaned and sterilized by immersing in mercuric bichlorid solution (1—1000) for an hour, and afterward rinsed in three changes of sterile distilled water. With a sterile scalpel each tuber was cut in two. One-half was inoculated from a 48-hour culture of the bacterium grown upon steamed potato plugs, while the other half of each potato was held as a check. Inoculation was made in the potato with a stiff platinum wire carrying the inoculum. Examination after 4 weeks showed that very slight bacterial growth had developed at the point of inoculation in each case to a depth of approximately 1 millimeter. There was no rotting of the inoculated portions and the checks remained disease free.

LONGEVITY IN SOIL CULTURES

In this connection it was deemed of practical value to determine whether the bacterium would remain alive in soil cultures for any considerable period. Such cultures were prepared by placing 150 grams of hothouse soil in each of three one-half liter flasks. These were sterilized daily for four successive days in an autoclave for two hours at 15 pounds pressure. Between sterilization periods the soil in each flask was thoroughly mixed by shaking. After an inoculation period of 24 hours following the last sterilization the soil in each flask was tested by placing samples removed by means of a stiff platinum loop upon nutrient agar plates. After a week all plates were found to be sterile.

The soil cultures were then inoculated from a four-day-old agar slant culture of the organism. The moisture of condensation rich in food material at the bottom of the slant was first drawn off with a sterile pipette. Approximately 5cc of sterile, physiological normal salt solution was then added and the bacterial growth washed free from the slant by gentle shaking. When a well-clouded suspension was obtained the liquid was drawn off, placed in a sterile flask and diluted with 10cc of the sterile salt solution. About 5cc of this suspension was added to each soil culture. These were afterwards weighed and set aside upon a shelf in the laboratory.

Examinations were made at intervals of four to six weeks throughout the year to ascertain whether the organism was still alive. This was done by removing 5 to 10 loopfuls of soil from each flask by means of a stiff platinum wire, and culturing upon potato or nutrient agar plates. The flasks were also weighed to determine the loss of water due to evaporation, and sterile distilled water added to compensate for the loss. The organism was recovered in each examination and the test demonstrated that the bacterium is able to survive in the soil for at least a year. About three months before the experiment was concluded one of the soil cultures became contaminated with a species of mucor. Even with the fungus present little difficulty was experienced in isolating the bacterium.

REACTION TO TEMPERATURE

Optimum temperature.—The temperature most favorable for growth is between 25 and 27 degrees C.

Maximum temperature.—In no case was growth observed to occur in cultures held at 38 degrees C. or above.

Minimum temperature.—Very slight growth occurred upon agar slant cultures incubated at 0 degrees C. A lower temperature is evidently necessary to arrest growth completely.

Thermal death point.—The thermal death point was determined to be between 51 and 52 degrees C. On two occasions there was growth in two tubes in a set of five when heated for 10 minutes at 52 degrees C., yet in no case did growth occur in all five tubes. Broth cultures 24 hours old were used for this test. Two methods were used. One consisted of placing 1cc of a broth culture in sterile thin-walled test tubes by means of a sterile pipette. These were heated in sets of five at temperatures varying from 47 to 56 degrees C. for 10 minutes. After heating, the tubes were incubated to determine viability, also transfers were made to sterile broth. In other cases two drops of a 24-hour broth culture were added to each tube of sterile nutrient broth, which was immediately heated and then incubated to determine viability.

BEHAVIOR TOWARD STAINS

With such stains as gentian violet, safranine, methylene blue, bismarck brown, dahlia, methyl violet, the organism stains uniformly and very quickly when aqueous, alcoholic, or aniline water solutions are used. It is not acid fast and does not stain by Gram's method.

DESIGNATION BY GROUP NUMBER

The cultural characteristics of the organism demonstrate the group number to be 211.3333523 in accordance with the descriptive chart of the Society of American Bacteriologists. When compared with the group number determined for *Bacterium vitians* Brown, the only point of distinction is noted to be that the Ohio organism reduces nitrates, whereas the South Carolina organism does not. Aside from the difference in action upon nitrates there is a marked difference in color of cultures of the two organisms of the same age upon the same media. The South Carolina organism produces spots upon the leaves and a rot of the stems of inoculated plants, while the one in Ohio has been found to be a soil loving parasite gaining entrance through the root system only, and causing a rosette type of growth. Furthermore, it has been found to be an active parasite of lettuce seedlings growing under excellent cultural conditions. The bacterium is therefore thought to be a new and distinct species and may be known as "Aplanobacter rhizoctonia n sp".

RETENTION OF VIRULENCE

The organism was found to retain its virulence for a year after growth upon artificial media also in soil cultures.

ABILITY TO WITHSTAND DRYING

The bacterium was tested for the purpose of determining its ability to resist drying. Drops as uniform as possible were placed upon sterile cover glasses which were kept in an incubator where the temperature varied from 25 to 30 degrees C. At the close of 24-hour periods one cover glass was removed and dropped in a flask of sterile bouillon. The test demonstrated that the organism will endure drying for four days. It was found to be dead after seven days.

RESUME OF TECHNICAL DESCRIPTION

Aplanobacter rhizoctonia n sp.—A short non-motile rod with rounded ends, becoming shorter as cultures age; pseudozoogloeae, but no capsules or endospores, strictly aerobic; agar colonies 24 hours old, greenish yellow, later olive buff, and finally after three to four days pyrite yellow; colonies round, entire, somewhat reticulate in structure, raised, not noticeably viscid. Growth good on all ordinary media, such as nutrient agar, oatmeal agar, nutrient broth, rice, potato plugs, and corn meal; also in peptone broth to which is added, dextrose, saccharose, maltose, lactose, mannit, or glycerine. It does not produce acid or gas; liquefies gelatin slowly. On all solid media color is at first

olive buff and later pyrite yellow. In milk the growth is good, the curd at first separates and later gradually disappears, the liquid becoming clear; after 11 days the color is olive buff, after 28 days coral pink, after 37 rufous, and after 38 coral red. This last color remains unchanged. It produces ammonia, and a good reduction of nitrates; the diastatic action on potato starch is noticeable yet not strong; there is slight production of indol; no hydrogen sulphid is produced; the growth in Uschinsky's and Fermi's solutions is good, but there is none in Cohn's; the color of Uschinsky's solution after 20 days is pinkish buff to vinaceous buff; thermal death point between 51 and 52 degrees C. maximum temperature for growth 38 degrees C., minimum below O degrees C., optimum 25 to 27 degrees C.; stains readily with all ordinary stains; is Gram negative, not acid fast; will live for at least a year in liquid media and in soil cultures and remain virulent; will grow in media containing 8 percent sodium chlorid; growth occurs in media made alkaline with sodium hydroxide to 20 degrees Fuller's scale, also in media containing acid as follows, malic +14 degrees, tartaric +20 degrees, lactic +12 degrees, citric +13 degrees, and acetic +17 degrees; not very sensitive to sunlight and stands dessication for four days.

ROUTINE METHOD OF ISOLATION

The procedure followed and established as a routine method for the isolation of the organism consisted in the removal of small pieces of tissue from the interior of diseased lettuce stems, with sterile instruments under asceptic conditions. These pieces were macerated in drops of sterile water in petri dishes and dilution plates made with potato or plain nutrient agar media. The cultures were incubated in the dark at a temperature varying from 25 to 30 degrees for 24 hours. They were next placed in the light at room temperature for 24 to 48 hours. Little difficulty was then experienced when examination was made for characteristic pyrite yellow colonies. Fairly satisfactory results were also obtained in many instances when 24-hour old cultures incubated in the laboratory outside the incubator were examined with a hand lens by focusing against a dark background. The colonies would appear as greenish yellow and very finely reticulate. Thinly sown check plates made from a known culture of the organism greatly facilitate the ease and accuracy of the work. Colonies so selected were ringed and later fished with a fine platinum wire, when transferred for isolation.

DIAGNOSTIC CHARACTERS

The following tests have been found to be fairly reliable for identification of the organism.

1. A rod-shaped organism not motile.
2. Colony circular, olive buff in color when incubated in the dark, pyrite yellow in subdued light.

3. Does not stain by Gram's method, not acid fast.
4. Gives good reduction of nitrates within 24 hours.
5. Growth good, not rapid, characteristic color constant on all ordinary media such as potato agar, plain nutrient agar, potato plugs, rice, oatmeal agar, etc.

CONTROL METHODS

The formaldehyde drench plan of soil sterilization was used on all beds containing diseased soils since means were not at our disposal to compare the relative merits of other methods. The soil

Bed of lettuce, 9 weeks old, grown in same bed as p.—. after the soil had been sterilized with formaldehyde

was first well loosened by thorough working. The formaldehyde solution, in proportion of 3 to 3½ pints or pounds to 50 gallons of water, was applied with a sprinkling can, at the rate of at least 1 gallon of liquid to each square foot of surface area. After treatment the beds were covered with canvas for 48 hours, which was then removed and the soil allowed to dry for a week or ten days. When the soil was sufficiently dried to be workable, the beds were prepared for planting and again set, together with the check beds, with five-week old lettuce plants. All beds developed uniformly, matured and were harvested at the same time.

SUMMARY

A bacterial rosette disease of lettuce has been found in a number of greenhouses in Ohio. This disease is thought to have been present for some time and has likely been confused with the older recognized form of rosette.

Inoculation experiments have demonstrated the pathogenicity of a bacterium which has, in every case, been associated with the disease. Furthermore, the organism has been consistently recovered from inoculated plants and its identity confirmed. The pathogene is thought to be able to survive in the soil indefinitely.

Studies of the organism in culture have revealed points of similarity and difference between it and another one known to cause a disease of lettuce in South Carolina, previously reported by Miss Nellie Brown of the United States Department of Agriculture. It is suggested that the Ohio organism may be known as "*Aplanobacter rhizoctonia n sp*".

The formaldehyde drench method was found to destroy the organism in the soil and is therefore suggested as a reliable control measure in case where it is not possible to employ steam sterilization.